Bulletin of the World Health Organization, **61** (1): 143-152 (1983)

Laboratory-based surveillance of influenza A(H1N1) and A(H3N2) viruses in 1980–81: antigenic and genomic analyses*

N. J. Cox,[1] Z. S. Bai,[2] & A. P. Kendal[3]

During 1981, the A/Brazil/11/78-like strains of influenza virus that had been prevalent from 1978 to 1980 were displaced by a new set of heterogeneous, but closely related, variants (reference strain, A/England/333/80). Genomic analysis revealed that these new variants were almost exclusively nonrecombinant H1N1 viruses, i.e., they contained no genes of H3N2 origin. However, a few recombinant viruses containing the new variant HA and genes of H3N2 origin were identified. Antigenic analysis of H3N2 viruses indicated that they were also heterogeneous. The majority of these virus isolates were antigenically intermediate between A/Texas/1/77 and A/Bangkok/1/79, but additional variants were detected. Genomic analysis revealed that the H3N2 viruses isolated in the winter of 1980–81 were quite similar to H3N2 viruses isolated from 1977–79 in their T_1 oligonucleotide maps. No H1N1 genes were detected in H3N2 virus isolates. Comparison of pairs of oligonucleotide maps of total virus RNA indicated that a similar rate of genetic change had occurred for nonrecombinant H1N1 viruses, for recombinant H1N1 viruses, and for H3N2 viruses and that, in general, pairs of viruses exhibited increasing numbers of changes in their oligonucleotide maps as the time interval between isolation of the viruses increased.

The remarkable re-emergence in 1977 of H1N1 viruses, antigenically and genetically similar to viruses that had circulated in 1950, has been well documented (*1–3*). Co-circulation of the newly emerged H1N1 viruses with the H3N2 viruses already in circulation (*4*) and the demonstration of mixed infections with both influenza A subtypes (*5*) prompted investigators to examine virus isolates for the presence of reassorted genomes. Conclusive evidence for the widespread circulation from 1978 to early 1980 of viruses containing four or five RNA segments from the H3N2 and the other three or four genes (including those coding for the haemagglutinin and neuraminidase antigens) from an H1N1 virus has been obtained in several laboratories (*6–8*; and J.F. Young, personal communication). The combined results of antigenic and genetic analysis led investigators to conclude that at least two separate recombinational events between H1N1 and H3N2 viruses must have occurred to account for the isolation of antigenically and genetically distinct H1N1 recombinant viruses (*9*). In the present study we again combined antigenic and genetic analyses of influenza A viruses in an investigation of the following questions:

— Do either recombinant or nonrecombinant H1N1 viruses have a consistent epidemiological advantage, as evidenced by their relative prevalence?

— Can additional recombinational events be detected for H1N1 or H3N2 viruses so that new combinations of genomes are present in viruses circulating since 1979?

— Is the emergence of new epidemic strains associated with a sudden overall genetic variation (detectable by oligonucleotide mapping) of total virion RNA

* From the WHO Collaborating Centre for Influenza, Division of Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, US Department of Health and Human Services, Atlanta, GA 30333, USA.

[1] Research Chemist, Influenza Branch, Centers for Disease Control.

[2] Director, Influenza Laboratory at the Lanzhou Institute of Biological Products, Lanzhou, Gansu Province, People's Republic of China.

[3] Chief, Influenza Branch, and Director, WHO Collaborating Centre for Influenza, Centers for Disease Control.

greater than that expected to arise in any given period of time?

— Do influenza viruses of different antigenic types exhibit different degrees of genetic heterogeneity?

MATERIALS AND METHODS

Viruses

The H1N1 and the H3N2 viruses used for this study are listed in Tables 2 and 4, respectively. They include previously described H1N1 and H3N2 prototype viruses, as well as additional variants detected in the 1980–81 season. These viruses were provided to the WHO Collaborating Center for Influenza at the Centers for Disease Control, Atlanta, GA, USA, by participants in the WHO global influenza surveillance programme. Viruses were grown in the allantoic cavity of 11-day-old embryonated eggs.

Haemagglutination-inhibition (HI) tests

These were carried out according to standard procedures using trypsin and periodate-treated postinfection ferret sera, or, in the case of monoclonal antibody analysis, using receptor-destroying enzyme-treated mouse ascitic fluids. Methods for preparation of ferret sera and ascitic fluids containing monoclonal antibodies directed specifically against the haemagglutinin of USSR/90/77 have been described previously (*10,11*).

Oligonucleotide fingerprinting

Viral RNA was extracted from purified egg-grown virions (*12*). Individual RNA segments were obtained from selected viruses by polyacrylamide gel electrophoresis (PAGE) of virion RNA on 3% polyacrylamide, 0.03 mol/l Tris-phosphate buffered (pH 7.8) slab gels or on 2.8% polyacrylamide, 0.08 mol/l Tris-borate buffered (pH 8.3) slab gels containing 6 mol/l urea. Conditions for PAGE were chosen to give maximum resolution of RNA segments. The gels were stained with a solution of 2 μg/ml ethidium bromide, segments were excised, and RNA was eluted overnight at 37 °C in buffer containing 0.5 mol/l ammonium acetate, 0.01 mol/l magnesium acetate, 0.1 mmol/l EDTA, and 0.1% SDS. Total RNA or RNA segments were digested with T_1 ribonuclease (Sankyo, Calbiochem), and the oligonucleotides were labelled at the 5' ends using γ^{32}P-ATP (adenosine triphosphate) (New England Nuclear Corporation) and T_4 polynucleotide kinase (Boehringer Mannheim), and were analysed essentially as already described (*9, 13*). For the two-dimensional mapping, electrophoresis in the first dimension was carried out in 10% polyacrylamide, 6 mol/l urea gels in 0.025 mol/l citric acid (pH 3.5) and in the second dimension was carried out at pH 8.3 in a 21.8% polyacrylamide gel buffered with Tris-borate.

RESULTS

Antigenic analysis of H1N1 influenza isolates

Previous studies, using ferret sera and monoclonal antibodies, identified six antigenic variants of A/USSR/90/77, of which one, A/Brazil/11/78, became the predominant strain in many regions of the world during 1978 and 1979 (*4, 11, 14*). Most of the variants from 1978 and 1979 resembled A/Brazil/11/78 in failing to react with at least the one A/USSR/90/77-specific monoclonal antibody, 264 (*15*). During 1980, isolates from several countries resembled A/Brazil/11/78 in their reaction patterns with ferret sera and the A/USSR/90/77 monoclonal antibodies. All viruses from outbreaks in England and India in the period April to August 1980, however, were found to differ from A/USSR/90/77 and A/Brazil/11/78 in their failure to react with an additional A/USSR/90/77-specific monoclonal antibody, 110 (Tables 1 and 2). The isolates from these outbreaks and epidemics were not homogeneous, but exhibited differences detectable with either ferret sera or monoclonal antibodies. Thus, A/England/333/80 represented a variant which cross-reacted well with A/Brazil/11/78, whereas A/India/6263/80 had reduced cross-reactions with A/Brazil/11/78. Nevertheless, the close relationship of A/India/6263/80 and A/England/333/80 was demonstrated by the strong reaction of A/India/6263/80 serum with A/England/333/80 virus. A further variant, represented by A/Plzen/26/80, was poorly inhibited by all sera; A/Plzen/26/80 serum reacted best with A/England/333/80 and A/India/6263/80, demonstrating the Plzen virus to be a poorly avid A/England/333/80-like strain (Table 1). Additional variants of the A/England/333/80-like virus (sometimes detected when samples of the same isolate, with different passage histories, were compared) could be demonstrated with monoclonal antibodies among viruses from England, India or other regions (*15*), but by the end of 1980 all H1N1 viruses tested failed to react with antibody 110 in addition to antibody 264. Thus, from 1978 to 1979, epidemic strains with a change in the epitope recognized by monoclonal antibody 264 displaced A/USSR/90/77 (e.g., virus with A/Brazil/11/78-like HA). In 1980, such viruses were displaced by new epidemic strains related to A/England/333/80 in which an additional epitope (recognized by monoclonal antibody 110) had undergone a fixed change.

Table 1. Haemagglutination-inhibition reactions of influenza A (H1N1) variants from 1980

Antigen	Ferret sera					A/USSR/77 monoclonal antibody				
	A/USSR/92/77[a]	A/Brazil/11/78	A/England/333/80	A/India/6263/80	A/Plzen/26/80	W18	22	70	110	264
A/USSR/90/77	320	320	640	20	640	3200	12800	25600	3200	6400
A/Brazil/11/78	80	640	640	20	640	1600	25600	25600	3200	< 100
A/England/333/80	160	640	1280	160	1280	1600	12800	12800	< 100	< 100
A/India/6263/80	40	80	160	320	320	200	12800	12800	< 100	< 100
A/Plzen/26/80	20	40	160	40	160	< 100	6400	6400	< 100	< 100

[a] Serum to recombinant with neuraminidase N7.

Genetic analysis of H1N1 influenza isolates

Representative H1N1 isolates from different areas of the world were analysed by T_1 oligonucleotide mapping of their virion RNA, and compared with prior reference strains representing nonrecombinant H1N1 viruses (A/USSR/90/77 and A/Brazil/11/78) and recombinant H1N1 viruses (A/California/10/78 and A/California/45/78) (*6–8*). Table 2 lists the viruses examined, along with their antigenic and genomic types. Throughout 1980, a few H1N1 viruses, typified by A/Singapore/25/80, were identified as recombinants by their similarity in oligonucleotide maps to A/California/10/78 (Fig. 1B and D). The H1N1 viruses containing recombinant genomes were not limited to those antigenically similar to A/Brazil/11/78; thus two isolates from China (Province of Taiwan) (Table 2 and Fig. IF), which had recombinant genomes, were characterized antigenically as having low avid A/England/333/80-like HA, similar to A/Plzen/26/80 (Tables 1 and 2). The majority of isolates examined, however, had T_1 oligonucleotide patterns typical of the nonrecombinant H1N1 type (Fig. 1C and E). Antigenically these viruses were either closely related to A/Brazil/11/78, or the more contemporary variant A/England/333/80. To rule out the possibility that the nonrecombinant H1N1 viruses contained a single H3N2 gene, we isolated segments of the A/India/6263/80 virus and compared oligonucleotide patterns directly with those of A/USSR/90/77 (H1N1) and with A/Texas/1/77 (H3N2) (data not shown). We were able to conclude that this representative H1N1 virus from 1980 contained no H3N2 virus genes. Thus, it appears that nonrecombinant H1N1 viruses were the predominant viruses circulating in the winter of 1980–81. Similar findings were made for viruses circulating in Japan (personal communication, S. Nakajima).

Antigenic analysis of H3N2 virus isolates

HI tests with post-infection ferret sera revealed that, as with the H1N1 virus isolates, a number of antigenic variants of H3N2 influenza were distinguishable. Table 3 lists the H3N2 variants in order of increasing drift from A/Texas/1/77. Viruses (like A/Oregon/4/80) that are highly cross-reactive with A/Texas/1/77 and A/Bangkok/1/79 were in the majority (Table 4, and unpublished results), although A/Bangkok/1/79, A/Bangkok/2/79 and other variants (e.g., A/Arizona/2/80 and A/Shanghai/31/80), with asymmetric differences from the major reference strains, were identified on several occasions (Tables 3 and 4). As observed above with the H1N1 viruses and previously with swine influenza viruses (*16*), it was not uncommon to identify several different variants from within an epidemic in one location.

Genetic analysis of H3N2 virus isolates

Representatives of most H3N2 antigenic variants from 1979–80 were examined by oligonucleotide mapping. In addition, we analysed the viruses A/England/864/75 (from a sporadic case (*17*)), A/Texas/1/77 (the probable index isolate from an outbreak on a military base (*18*)), and A/Alaska/6/77 (from an airplane-associated outbreak (*19*)). All these viruses, which were isolated before the re-emergence of H1N1 strains in 1977, are antigenically closely related to each other, and sequence analysis

Table 2. Antigenic and genetic types of representative influenza A(H1N1) viruses from 1980–81

Virus strain	Isolation date	HA type[a]	Genotype[b]
A/Maryland/1/80	1/80	A/Brazil/11/78	Nonrecombinant
A/Victoria/1/80	1/80	variant[c]	Nonrecombinant
A/Texas/1/80	2/80	A/Brazil/11/78	Recombinant
A/Texas/3/80	3/80	A/Brazil/11/78	Nonrecombinant
A/Santiago/228/80	5/80	A/Brazil/11/78	Nonrecombinant
A/Ecuador/4104/80	5/80	A/Brazil/11/78	Nonrecombinant
A/India/6263/80	5/80	1980 ref. strain	Nonrecombinant
A/India/6310/80	5/80	A/England/333/80	Nonrecombinant
A/India/6333/80	7/80	A/England/333/80	Nonrecombinant
A/India/6587/80	8/80	A/England/333/80	Nonrecombinant
A/Peru/394/80	8/80	A/Brazil/11/78	Recombinant
A/Singapore/22/80	8/80	A/Brazil/11/78	Recombinant
A/Singapore/25/80	8/80	A/Texas/23/79	Recombinant
A/Puerto Rico/1/80	9/80	A/Brazil/11/78	Recombinant
A/England/403/80	11/80	A/India/6263/80	Nonrecombinant
A/England/435/80	11/80	A/England/333/80	Nonrecombinant
A/Lerwick/61694/80	11/80	variant[d]	Nonrecombinant
A/Singapore/39/80	11/80	A/England/333/80	Nonrecombinant
A/Texas/22/80	12/80	A/India/6263/80	Nonrecombinant
A/Texas/23/80	12/80	A/England/333/80	Nonrecombinant
A/Washington DC/101/80	12/80	A/England/333/80	Nonrecombinant
A/Washington DC/102/80	12/80	A/England/333/80	Nonrecombinant
A/Memphis/7/80	12/80	A/England/333/80	Nonrecombinant
A/Israel/1/80	12/80	A/Brazil/11/78	Nonrecombinant
A/Hungary/70/80	12/80	A/Brazil/11/78	Nonrecombinant
A/Prague/57/80	12/80	A/Brazil/11/78	Nonrecombinant
A/Ostrova/12/80	12/80	A/India/6263/80	Nonrecombinant
A/Plzen/26/80	12/80	1980–81 ref. strain	Nonrecombinant
A/Taiwan/740/80	12/80	A/Plzen/26/80	Recombinant
A/Taiwan/777/80	12/80	A/Plzen/26/80	Recombinant
A/Fukuoka/9/81	2/81	A/Plzen/26/80	Nonrecombinant
A/Taiwan/709816/81	7/81	A/England/333/80	Nonrecombinant
A/Uruguay/1/81	10/81	A/India/6263/80	Nonrecombinant

[a] Determined by HI testing with ferret sera and monoclonal antibodies. See Table 1 for antigenic specificities.
[b] Determined by comparing T_1 oligonucleotide maps of virion RNA to maps of known recombinant and nonrecombinant viruses.
[c] Variant from A/Brazil/11/78 (*15*).
[d] Variant from A/England/333/80 (*15*).

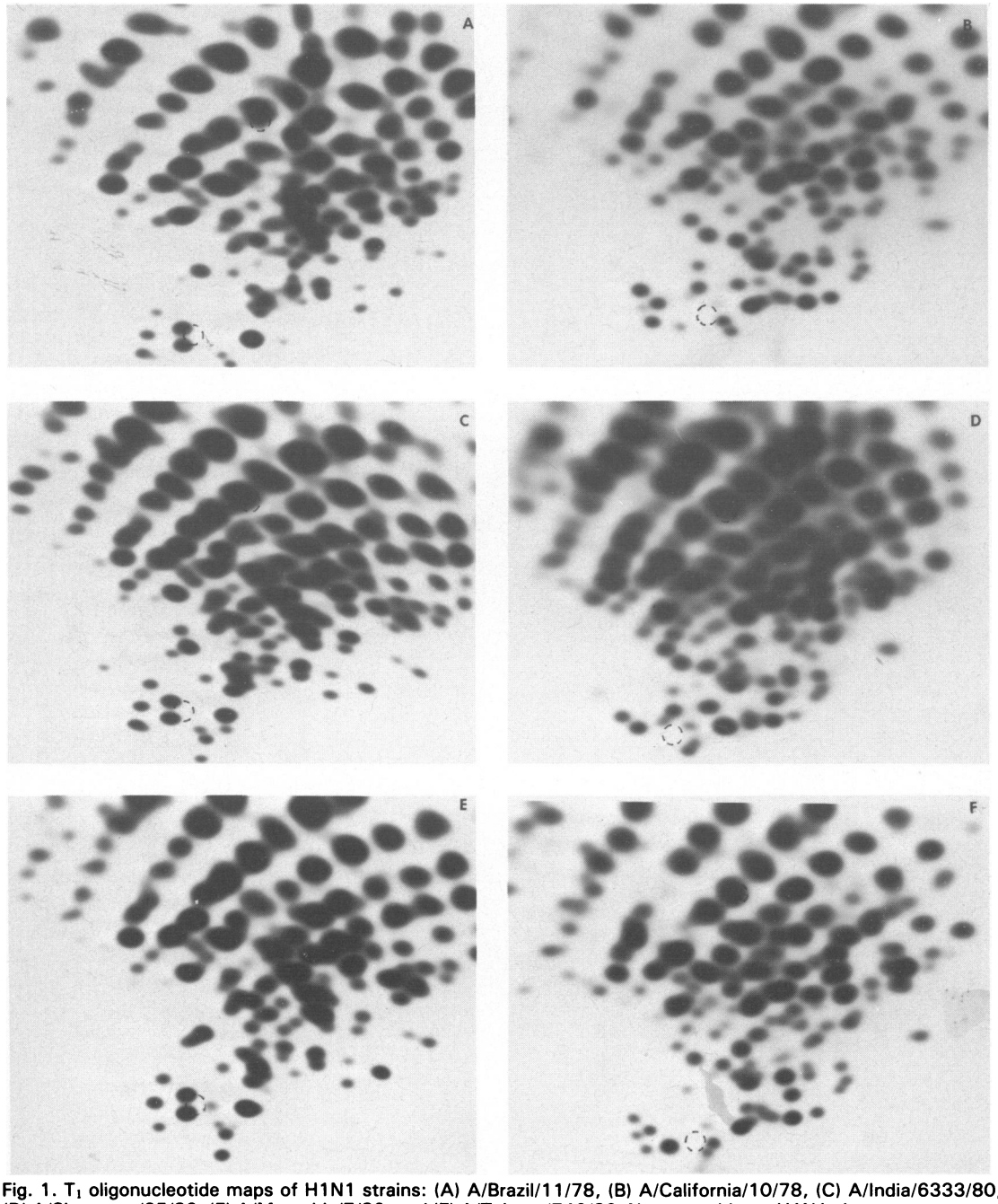

Fig. 1. T_1 oligonucleotide maps of H1N1 strains: (A) A/Brazil/11/78, (B) A/California/10/78, (C) A/India/6333/80, (D) A/Singapore/25/80, (E) A/Memphis/7/80, and (F) A/Taiwan/740/80. Nonrecombinant H1N1 viruses are grouped on the left (A, C, and E) and recombinant H1N1 viruses are grouped on the right (B, D, and F). Total RNAs extracted from egg-grown purified virions were digested with T_1 ribonuclease. The resulting oligonucleotides were labelled at the 5′-end with (γ^{32}P)-adenosine triphosphate using polynucleotide kinase and separated by two-dimensional polyacrylamide gel electrophoresis. The first dimension (left to right) was at pH 3.5 in a 10% polyacrylamide, 6 mol/l urea gel, and the second dimension (bottom to top) was at pH 8.3 in a 21.8% polyacrylamide gel buffered with Tris-borate. The positions of the dye markers are shown by the circles (the lower one is xylene cyanol FF and the upper one is bromophenol blue).

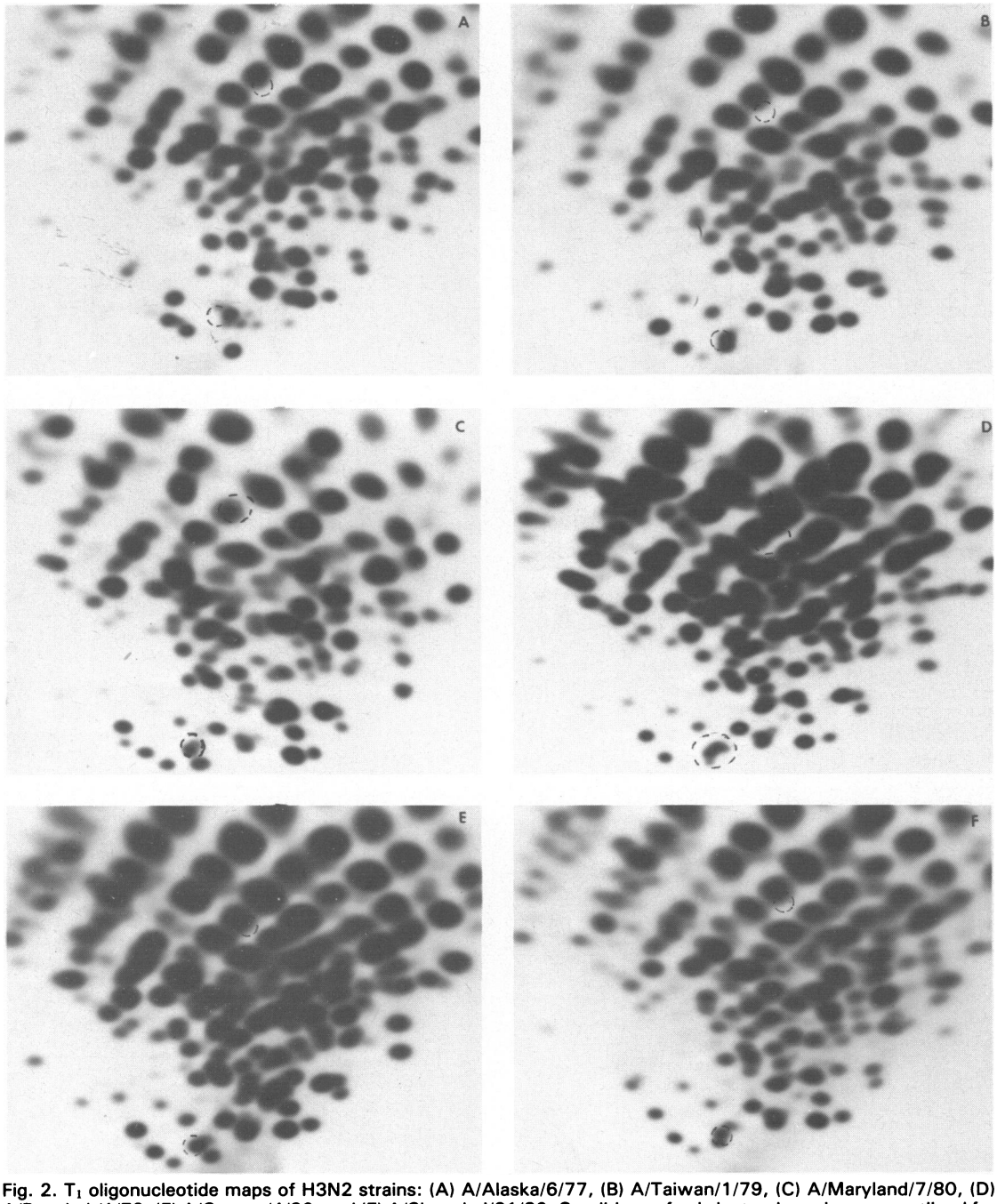

Fig. 2. T_1 oligonucleotide maps of H3N2 strains: (A) A/Alaska/6/77, (B) A/Taiwan/1/79, (C) A/Maryland/7/80, (D) A/Bangkok/1/79, (E) A/Oregon/4/80, and (F) A/Shanghai/31/80. Conditions of gel electrophoresis are as outlined for Fig. 1.

Table 3. Haemagglutination-inhibition reactions of influenza A (H3N2) variants from 1979–80

	Ferret sera					
Antigen	A/Texas/1/77[a]	A/Oregon/4/80[a]	A/Bangkok/1/79[a]	A/Arizona/2/80	A/Bangkok/2/79	A/Shanghai/31/80[a]
A/Texas/1/77	2560	640	160	640	80	160
A/Oregon/4/80	1280	1280	640	1280	320	640
A/Bangkok/1/79	640	640	1280	640	320	2560
A/Arizona/2/80	640	320	160	640	160	320
A/Bangkok/2/79	320	320	160	320	2560	1280
A/Shanghai/31/80	80	160	320	160	80	1280

[a] Serum to recombinant with neuraminidase N7.

shows that their haemagglutinin is the precursor of those in the A/Bangkok/79-related strains that circulated in 1979–81 (Both, Sleigh & Kendal, unpublished results). This is supported by the results of oligonucleotide mapping which showed a high degree of similarity for the total genome of H3N2 isolates from 1977–80 (Fig. 2).

To examine the possibility that recombinants bearing the surface antigens of the H3N2 subtype but containing some genes of H1N1 origin might exist, we completed T_1 oligonucleotide maps for individual segments of the A/Texas/1/77 and A/USSR/90/77 viruses and compared these patterns with the total virion RNA pattern of the 1979 and 1980 H3N2 viruses. No evidence was obtained for the presence of H1N1 genes in any of the viruses analysed (data not shown). This is consistent with the results obtained by RNA–RNA hybridization for a few H3N2 isolates from 1978–79 that were tested previously (*7*).

Analysis of oligonucleotide heterogeneity among RNAs of influenza viruses

We have examined a relatively large number of H1N1 and H3N2 viruses isolated over a 3-year period and having a broad geographical distribution. By means of pairwise comparisons between T_1 maps of virus isolates, we were able to quantitate the numbers of detectable oligonucleotide changes between total viral RNAs of virus isolates from the same or different years, and with similar or dissimilar antigenic

Table 4. Antigenic types of representative influenza A(H3N2) viruses from 1980–81

Virus strain	Date of isolation	HA type[a]
A/Guan Dong/22/80	5/80	A/Bangkok/2/79
A/Guan Dong/30/80	5/80	A/Bangkok/1/79
A/Bangkok/38/80	10/80	A/Shanghai/31/80
A/Alaska/3/80	10/80	A/Arizona/2/80
A/Arizona/11/80	11/80	A/Oregon/4/80
A/Arizona/12/80	11/80	A/Oregon/4/80
A/San Francisco/5/80	11/80	A/Oregon/4/80
A/Nevada/2/80	11/80	A/Arizona/2/80
A/Texas/10/80	11/80	A/Oregon/4/80
A/Canada/446/80	11/80	A/Bangkok/1/79
A/Canada/506/80	11/80	A/Bangkok/2/79
A/Berkeley/3/80	11/80	A/Oregon/4/80
A/Shanghai/31/80	12/80	1980–81 ref. strain[b]
A/Georgia/1/80	12/80	A/Oregon/4/80
A/Oregon/4/80	12/80	1980–81 ref. strain[c]
A/Maryland/7/80	12/80	A/Oregon/4/80
A/New Jersey/8/80	12/80	A/Bangkok/2/79
A/Brazil/45/81	2/81	A/Oregon/4/80

[a] Determined by HI testing with ferret sera. See Table 3 for antigenic specificities.

[b] Drift variant of A/Bangkok/1/79.

[c] Cross-reactive with A/Texas/77 and A/Bangkok/1/79.

types. Viruses of a given genotype (recombinant or nonrecombinant), closely related in time and place of isolation, exhibited a few (1-8) oligonucleotide differences. Viruses more distantly related in time or in place of isolation exhibited a proportionately greater number of oligonucleotide differences (Table 5). The occurrence of major epidemics (e.g., by H3N2 viruses in the USA in 1980-81) was not associated with the appearance of influenza virus isolates which had an unexpectedly large number of oligonucleotide changes in their RNAs (e.g., A/Oregon/4/80 in Fig. 2).

Table 5. Oligonucleotide differences between pairs of influenza viruses

Comparison of pairs (virus subtype)	Time interval of isolation (months)	Average no. of spot differences (range)	No. examined
H3N2	< 12	5 (1-8)	14
	12-23	6 (5-7)	6
	24-35	10 (8-13)	5
	36-47	14 (11-17)	5
H1N1 nonrecombinant	< 12	4 (2-8)	9
	12-23	9 (7-10)	12
	24-35	11 (10-13)	12
H1N1 recombinant	< 12	4 (2-7)	9
	12-23	9 (7-10)	4
	24-35	11 (10-12)	4

DISCUSSION

One objective of this study was to determine the relative prevalence of nonrecombinant and recombinant H1N1 viruses over a longer time period than previously studied. We have shown that both genotypes of H1N1 viruses could be isolated from a given location within a short time (e.g., A/Texas/1/80 and A/Texas/3/80; A/Singapore/25/80 and A/Singapore/39/80). Furthermore, based on the genome composition of influenza virus isolates submitted from different regions of the world where influenza morbidity had been reported in 1980-81, we can conclude that the recombinant H1N1 viruses do not appear to have a long-term selective advantage over the nonrecombinant H1N1 viruses. Thus, nearly all the new antigenic variants of H1N1 influenza (related to A/England/333/80), which became predominant in 1980, had nonrecombinant genomes. Exceptions were the H1N1 viruses from China (province of Taiwan), which had recombinant genomes, but HA related to that of post-A/Brazil/11/78 variants. The Taiwan viruses may have resulted from recombination between a contemporary H1N1 virus and a virus containing H3N2 genes or, alternatively, may have evolved independently by antigenic drift from a precursor recombinant H1N1 strain with A/Brazil/11/78-like HA. The sequence of prevalence of different H1N1 viruses in the USA from 1977 to 1981 is summarized in Table 6. A generally similar sequence is believed to have been followed worldwide, but with some regional differences (e.g., recombinant H1N1 viruses were not found among the tested isolates from Australia or India).

Second, we found no evidence for the presence of recombinant genomes in the H3N2 viruses examined, though theoretically these would occur with the same frequency as recombinant H1N1 viruses after mixed H1N1-H3N2 infection. Results presented are limited

Table 6. Detection of influenza A(H1N1) virus with different antigenic and genomic compositions in the USA during 1977-81

Period	HA	Recombinant genome	Prevalence
May 1977-April 1978	A/USSR/90/77	-	Epidemic
	A/Brazil/11/78	-	Sporadic
May 1978-April 1979	A/USSR/90/77	+	Sporadic
	A/Brazil/11/78	+	Epidemic
May 1979-April 1980	A/Brazil/11/78	+	Sporadic
	A/Brazil/11/78	-	Outbreaks
May 1980-April 1981	A/Brazil/11/78	-	Outbreaks
	A/England/333/80	-	Epidemic

by the fact that, for most field strains tested, total genomic RNA is examined and only a limited proportion of the genome is screened by this technique. Furthermore, oligonucleotide mapping is biased toward the large genome segments, because they provide many of the larger T_1 oligonucleotides resolved in the fingerprint of total RNA. Therefore, we cannot exclude the possibility that the smallest RNA segments of an individual isolate may have been derived from a different subtype than were its other genes.

A further objective of the study was to compare the genetic heterogeneity observed between epidemic strains and within groups of viruses having similar genomes. We observed similar genetic heterogeneity within each virus genotype (nonrecombinant H1N1 viruses, H3N2 viruses, and H1N1–H3N2 recombinant viruses). This suggests that there was a comparable selective pressure and fidelity of replication for each virus genotype, so that genetic variation detectable by oligonucleotide mapping was primarily dependent on the time interval between isolation of viruses. Nucleic acid sequencing has shown that the rate of base-sequence changes per year may vary from approximately 0.12% for the matrix protein (*20*) to approximately 0.54% for the haemagglutinin gene (*21*, *22*). While oligonucleotide mapping is a less accurate method for quantifying changes, it has been shown to provide estimates of base-sequence differences between RNAs that correlate well with values obtained from sequencing data (*20*). Assuming that we are analysing 10% of the viral genome (approximately 1400 bases) in the 65 or so large oligonucleotides compared, and that each spot difference represents a change, we estimate the rate of change to be about 0.3–0.6% per year, which is consistent with the rates of evolution determined by sequencing studies.

No significant increase in the number of oligonucleotide spot differences (greater than that expected on the basis of the time interval of isolation) was observed with the emergence of a new variant subtype, and oligonucleotide heterogeneity did not appear to correlate with antigenic specificity or epidemic activity. These observations emphasize that oligonucleotide mapping cannot be used to predict the epidemic potential of a new influenza strain, but rather is a useful technique for the identification of strains requiring further study because of an unusual genetic composition. The base-sequence variation detected by oligonucleotide mapping in influenza viruses, even within epidemics, parallels the detection of polymorphism in the HA detected by antigenic analysis. Theories about the evolution of new epidemic variants need to account for these facts and should not assume that epidemic strains are genetically or antigenically uniform.

ADDENDUM

Since this text was prepared, antigenic and genomic analyses of two influenza A(H1N1) viruses isolated in China (province of Taiwan) in 1982 revealed them to be A/England/333/80- and A/India/6263/80-like viruses and to have non-recombinant genomes.

ACKNOWLEDGMENTS

Z.S. Bai was the recipient of a fellowship provided by the World Health Organization.

We thank the many laboratories participating in the WHO influenza surveillance programme that sent influenza viruses for reference analysis. We also thank Dr R.G. Webster for the gift of A/USSR/90/77 monoclonal antibodies and Ms Connie Carmack who provided expert technical assistance.

RÉSUMÉ

SURVEILLANCE EN LABORATOIRE DES VIRUS GRIPPAUX A(H1N1) ET A(H3N2) EN 1980–1981: ANALYSE DES ANTIGÈNES ET DU GÉNOME

En 1981, les souches de virus grippal analogues à A/Brazil/11/78 qui avaient été prévalentes de 1978 à 1980 ont été supplantées par un nouvel ensemble de variants hétérogènes, mais étroitement apparentés (souche de référence A/England/333/80). L'analyse du génome a révélé que ces nouveaux variants étaient presque exclusivement des virus H1N1 non recombinants, c'est-à-dire ne contenant aucun gène d'origine H3N2. Toutefois, quelques virus recombinants contenant le nouveau variant HA et des gènes d'origine H3N2 ont été identifiés. L'analyse antigénique des virus H3N2 a indiqué qu'ils étaient également hétérogènes. La plupart de ces isolements de virus étaient antigénique-

ment intermédiaires entre A/Texas/1/77 et A/Bangkok/1/79, mais d'autres variants ont été détectés. L'analyse du génome a révélé que les virus H3N2 isolés au cours de l'hiver 1980–1981 étaient tout à fait analogues aux virus H3N2 isolés en 1977–1979 en ce qui concerne la carte des oligonucléotides T_1. Aucun gène de H1N1 n'a été décelé dans les isolements de virus H3N2. La comparaison par paires des cartes d'oligonucléotides de l'ARN viral total a indiqué qu'un taux analogue de modifications génétiques avait eu lieu pour les virus H1N1 non recombinants, les virus H1N1 recombinants et les virus H3N2 et qu'en général les paires de virus présentaient un nombre croissant de modifications des cartes d'oligonucléotides à mesure que le délai avant isolement des virus augmentait.

REFERENCES

1. WORLD HEALTH ORGANIZATION. Influenza in the world, October 1977–September 1978. *Weekly epidemiological record*, **54**: 25 (1979).
2. SCHOLTISSEK, C. ET AL. Genetic relatedness between the new 1977 epidemic strains (H1N1) of influenza and human influenza strains isolated between 1947 and 1957. *Virology*, **86**: 613–617 (1978).
3. NAKAJIMA, K. ET AL. Recent human influenza A (H1N1) viruses are closely related genetically to strains isolated in 1950. *Nature (London)*, **274**: 334–339 (1978).
4. KENDAL, A. P. ET AL. Laboratory-based surveillance of influenza virus in the United States during the winter of 1977–1978. I. Periods of prevalence of H1N1 and H3N2 influenza A strains, their relative rates of isolation in different age groups, and detection of antigenic variants. *American journal of epidemiology*, **110**: 449–461 (1979).
5. KENDAL, A. P. ET AL. Laboratory-based surveillance of influenza virus in the United States during the winter of 1977–1978. II. Isolation of a mixture of A/Victoria-and A/USSR-like viruses from a single person during an epidemic in Wyoming, USA, January 1978. *American journal of epidemiology*, **110**: 462–468 (1979).
6. YOUNG, J. F. & PALESE, P. Evolution of human influenza A viruses in nature: recombination contributes to genetic variation of H1N1 strains. *Proceedings of the National Academy of Sciences (USA)*, **76**: 6547–6551 (1979).
7. BEAN, W. J. ET AL. Recombination of human influenza A viruses in nature. *Nature (London)*, **284**: 638–640 (1980).
8. NAKAJIMA, S. ET AL. Influenza surveillance based on oligonucleotide mapping of RNA of H1N1 viruses prevalent in Japan, 1978–1979. *Journal of infectious diseases*, **142**: 492–502 (1980).
9. NAKAJIMA, S. ET AL. Antigenic and genomic analyses of influenza A (H1N1) viruses from different regions of the world, February 1978 to March 1980. *Infection and immunity*, **32**: 287–294 (1981).
10. DOWDLE, W. R. ET AL. Influenza viruses. In: Lennette, E. H. & Schmidt, N. J., (ed.) *Diagnostic procedures for viral, rickettsial and chlamydial infections*. American Public Health Association, New York, 1979, pp. 585–609.
11. WEBSTER, R. G. ET AL. Analysis of antigenic drift in recently isolated influenza A (H1N1) viruses using monoclonal antibody preparations. *Virology*, **86**: 258–264 (1979).
12. PALESE, P. & SCHULMAN, J. L. Differences in RNA patterns of influenza A virus. *Journal of virology*, **17**: 876–884 (1976).
13. PEDERSON, F. S. & HASELTINE, W. A. A micro-method for detailed characterization of high molecular weight RNA. *Methods in enzymology*, **65**: 680–687 (1980).
14. KENDAL, A.P. ET AL. Effect of test system on the ability of monoclonal antibodies to detect antigenic drift in influenza A (H1N1) virus hemagglutinins. *Journal of general virology*, **54**: 253–261 (1981).
15. KENDAL, A. P. ET AL. Natural and unnatural variation in influenza A (H1N1) viruses since 1977. In: Nayak, D & Fox, F., (ed.) *Genetic variation among influenza viruses*. ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. XXII, New York, Academic Press, 1981, pp. 489–504.
16. KENDAL, A. P. ET AL. Swine influenza viruses isolated in 1976 from man and pig contain two coexisting subpopulations with antigenically distinguishable hemagglutinins. *Virology*, **82**: 111–121 (1977).
17. WORLD HEALTH ORGANIZATION. Influenza in the world, October 1975–September 1976. *Weekly epidemiological record*, **52**: 37–44 (1977).
18. CENTERS FOR DISEASE CONTROL. Influenza Surveillance Report No. 92, January 1981. Summary, September 1976–June 1977.
19. MOSER, M. R. ET AL. An outbreak of influenza abroad a commercial airliner. *American journal of epidemiology*, **110**: 1–6 (1979).
20. HALL, R. M. & AIR, G. M. Variation in nucleotide sequences coding for the N-terminal regions of the matrix and nonstructural proteins of influenza virus. *Journal of virology*, **38**: 1–7 (1981).
21. VERHOEGEN, M. ET AL. Antigenic drift between the hemagglutinin of the Hong Kong influenza strains A/Aichi/2/68 and A/Victoria/3/75. *Nature (London)*, **286**: 771–776.
22. AIR, G. M. Sequence relationships among the subtypes of influenza A virus. *Proceedings of the National Academy of Sciences (USA)*, **78**: 7639–7643 (1981).
23. YOUNG, J. F. ET AL. Advantages and limitations of the oligonucleotide mapping technique for the analysis of viral RNAs. In: Bishop, D. H. L. & Compans, R. W., ed. The replication of negative strand viruses. *Developments in cell biology*. Vol. 7, 1981, pp. 209–215.